PRICK TESTING IN INSECT BITE REACTION

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In fulfilment of the requirements for the award of the degree of

Doctor of Medicine in Dermatology, Venereology and Leprology

Under the guidance of

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THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, TAMILNADU

MAY 2018

CERTIFICATE

This is to certify that the thesis entitled **"PRICK TESTING IN INSECT BITE REACTION"** is a bonafide work of **Dr. IYSHWARIYA SIVADASAN** done under the direct guidance and supervision of **Dr.C.R. SRINIVAS, MD** and **Dr. SHANMUGA SEKAR .C, MD,** in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of The Tamil Nadu Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

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I hereby declare that this dissertation entitled **"PRICK TESTING IN INSECT BITE REACTION"** was prepared by me under the direct guidance and supervision of **Dr.C.R.SRINIVAS, MD** and **Dr. SHANMUGA SEKAR C., MD,** PSG Institute of Medical Sciences and Research, Coimbatore.

The dissertation is submitted to The Tamil Nadu Dr. MGR Medical University in fulfillment of the University regulation for the award of MD degree in Dermatology, Venereology and Leprology. This dissertation has not been submitted for the award of any other Degree or Diploma.

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This is to certify that the thesis entitled **"PRICK TESTING IN INSECT BITE REACTION"** is a bonafide work of **Dr. IYSHWARIYA SIVADASAN** done under my direct guidance and supervision in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of The Tamil Nadu Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

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CERTIFICATE – II

This is to certify that this dissertation work titled **PRICK TESTING IN INSECT BITE REACTION** of the candidate **Dr. Iyshwariya Sivadasan,** with registration Number **201530351** for the award of **Doctor of Medicine** in the branch of **Dermatology**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** of plagiarism in the dissertation.

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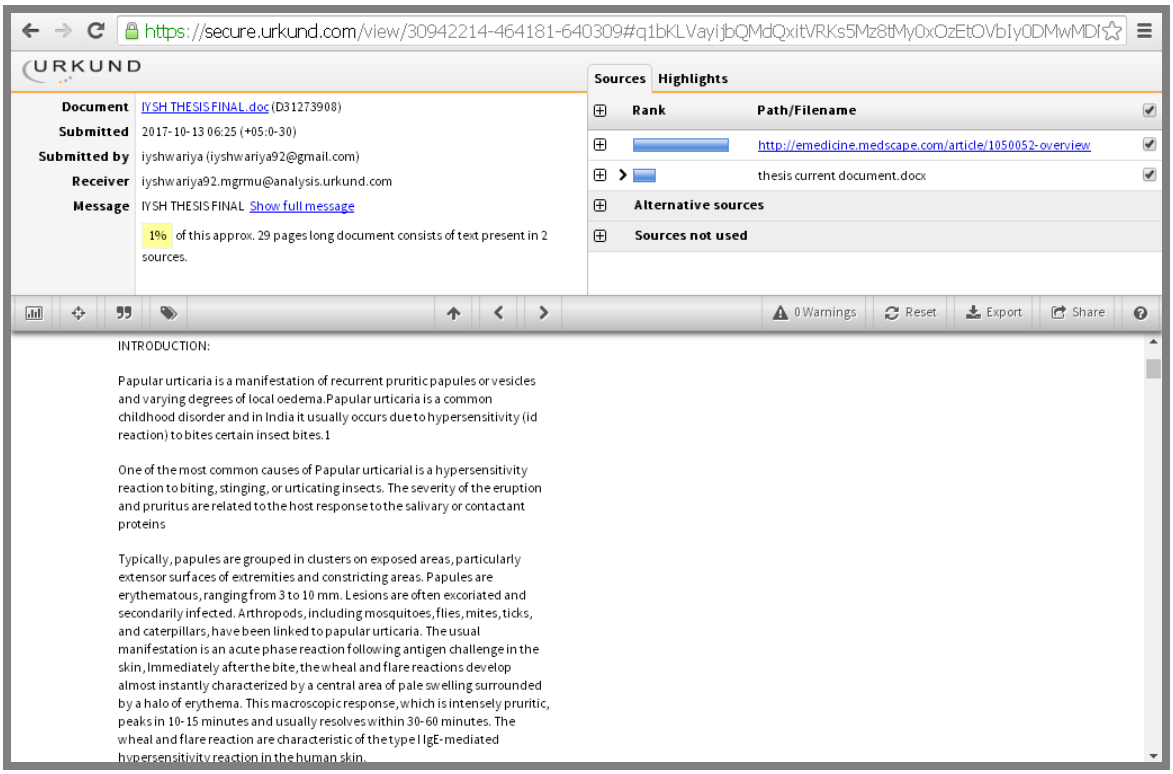

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INTRODUCTION

Papular urticaria is a manifestation of recurrent pruritic papules or vesicles and varying degrees of local oedema.Papular urticaria is a common childhood disorder and in India it usually occurs due to hypersensitivity (id reaction) to certain insect bites.[1]

One of the most common causes of Papular urticarial is a hypersensitivity reaction to biting, stinging, or urticating insects. The severity of the eruption and pruritus are related to the host response to the salivary or contactant proteins

Typically, papules are grouped in clusters on exposed areas, particularly extensor surfaces of extremities and constricting areas. Papules are erythematous, ranging from 3 to 10 mm. Lesions are often excoriated and secondarily infected. Arthropods, including mosquitoes, flies, mites, ticks, and caterpillars, have been linked to papular urticaria.

The usual manifestation is an acute phase reaction following antigen challenge in the skin. Immediately after the bite, the wheal and flare reactions develop almost instantly characterized by a central area of pale swelling surrounded by a halo of erythema. This macroscopic response, which is intensely pruritic, peaks in 10-15 minutes and usually resolves within 30-60 minutes. The wheal and flare reaction are characteristic of the type I IgE-mediated hypersensitivity reaction in the human skin.

But there are some studies demonstrating the occurrence of a late phase reaction to these insect bites peaking at about 6 to 8 hours and resolved within 24 hours. [2] The mechanism responsible for this phenomenon is not yet clear.

Limited evidence based on biopsy specimens from the skin lesion have demonstrated strong IgE mediated inflammatory response in these late phase lesions.[3] But on further observation for longer periods has shown that in many instances a late inflammatory response appears at the same site and is quite different in appearance from the initial reaction.

NEED FOR THE STUDY

Even though there is an abundance of literature focusing on the early phase reaction in papular urticaria, the number of studies on late phase reaction are relatively rare. Since the possibility of an inflammatory response different from IgE mediated hypersensitivity reaction has been postulated and this may have strong implications for choice of treatment. While the early phase reactions are known to be effectively controlled by anti-histaminic medication, the late phase reaction may probably need treatment with steroid therapy.

AIM AND OBJECTIVES

AIM

The study was conducted with an aim to assess the pattern of late phase response to various antigens in the insect series in patients with papular urticaria

OBJECTIVES

To assess the type 1 hypersensitivity reaction – early phase (assessed at 15 minutes) and late phase (assessed at 6 hours) by prick testing with insect series in patients with papular urticaria.

REVIEW OF LITERATURE

EPIDEMIOLOGY OF INSECT BITES IN INDIA:

Arthropods, including mosquitoes, flies, gnats, mites, ticks, and caterpillars, have been linked to papular urticaria. These organisms belong to *Arthropoda*, "joint-footed animal," is the largest phylum in the animal kingdom. It accounts for approximately 80% of the animals and encompasses more than 1.5 million described species. There multiple classes among arthropods which are associated with papular urticaria. The predominant classes being Insecta and Arachnida.

Hexapoda / Insecta (Insects):

The class Hexapoda (true insects) have the following characteristic features.

- Three pairs of legs
- Three distinct body segments: the head, the thorax, and the abdomen.
- One or two pairs of wings in some insects

This class includes most of the arthropods responsible for adverse reactions, particularly immediate hypersensitivity. Although insects can precipitate direct envenomation effects, significant toxicity is usually associated with multiple stings or bites. Those who work outdoors and those who are involved in outdoor sports or activities find that they have to share their activity with a variety of insects. The most important insects associated with papular urticaria in India are Mosquitoes, Flies, and midges, Fleas, Lice, caterpillar, and beetles etc.

MOSQUITOES

Mosquitoes, like other members of the class Diptera, have one pair of front wings, with a hind pair of small, knobbed structures referred to as halters. They require a blood meal during some stage of their development.

There are more than 2500 species of mosquitoes. It is estimated that more than 1 million people are bitten by mosquitoes daily. Although there have not been any reported cases of death attributed to hypersensitivity to mosquitoes, there are numerous reports of cutaneous allergic reactions.

In addition to adverse reactions to mosquito saliva, there are also reports of inhalant allergy to the scale. Saliva of mosquitoes contains pharmacologically active compounds inhibiting body's innate immune responses, causing anticoagulation, impaired platelet formation, vasodilation and anti-inflammatory activities. [4] Allergens in mosquito whole body extract and saliva have been studied for developing diagnostic tests and immunotherapy for mosquito bite allergy.

These approaches are used infrequently and mosquito whole body extracts are ineffective in down regulating specific immune responses to salivary allergens and may enhance sensitization. Salivary gland surface proteins are major immunogenic components.[5]

Mellanby [6] in 1946 in his article titled "man's reaction to mosquito bite" has described 5 stages of reaction to repeated mosquito bites

Stage 1: No observable reaction, period of induction of hypersensitivity

Stage 2: Pruritic papules appear after about 24 hours of bite and lasting for several days

Stage 3: Delayed papule, in addition to developing an immediate wheal lasting for a few hours

Stage 4: Development of only immediate wheal without delayed papule

Stage 5: Complete tolerance with no reaction

FLIES AND MIDGES

Many species of flies and midges bite humans.

Black flies (*Simuliidae* spp.) are considered to be one of the most intolerable pests that bite humans. The bite of a black fly is initially painless, because of a topical anesthetic it secretes. Eventually, the site of the bite becomes painful, erythematous, and pruritic, developing into vesicles and edematous papules. The bite also causes a systemic reaction, with nausea, vomiting, malaise, and generalized lymphadenopathy. Black flies also are important vectors for tularemia and onchocerciasis (river blindness).

Horseflies and deerflies[7] are the most common of the 3000 species of flies in the Tabanidae family. They bite viciously and deeply, resulting in immediate pain, bleeding, and, often, subsequent local infection. Bites are single, although multiple bites have been reported. Additionally, horsefly bites might induce a wheal and flare response and have also been associated with more severe systemic symptoms. More than 30 cases of allergic reactions to horsefly bites have been reported Cutaneous myiasis is the infestation of tissue by the larvae of flies.

Flies lay their eggs on the surface of the skin. The eggs hatch and the larvae burrow into the tissue, leaving a central hole to provide air. Infestation is caused by many fly species. Wound myiasis begins with flies laying eggs on open

wounds. Once the eggs hatch, the larvae penetrate the skin and begin feeding on the necrotic tissue.

In the case of the botfly, the eggs hatch on another insect and penetrate the skin of the victim while the insect takes its blood meal. Intense pruritus is often the presenting symptom.

As the larvae grow, an erythematous, edematous papule forms, with resultant induration. Within a few days, the wound develops serosanguinous drainage from the central hole. This drainage becomes more prominent as the maggots grow.

Treatment focuses on the removal of the larvae, intact, by surgical excision. Occlusion of the central hole, asphyxiating the larvae, might be effective

Two patients with Simulium dermatitis from North- Eastern region of India had intense itching, excoriations, scarring, and hyperpigmentation. Histopathology showed vesicles, dermal oedema, and perivascular infiltrates rich in eosinophils and lymphocytes. [8]

There are other varieties of flies including Blandford Flies, the bites of which produce skin swellings and sometimes fever or joint pain.[9] is another fly species of dermatological significance.

Louse flies a hematophagous louse fly of deer, causes pruritic papules, usually in forests. The Lesions caused by louse flies appear mostly on head and back, are resistant to treatment and persist for weeks to months.

Direct immunofluorescence may show deposits of C3 in dermal vessel walls. IgE, complement and cell-mediated mechanisms are involved.[10]

Tsetse flies another important category of flies which includes all species in genus *Glossina*, generally placed in their own family, Glossinidae. They are reported to be confined to only African regions and not yet reported from India.[11]

Midges

Biting midges, known as "no-see-ums," are a common nuisance. The 1- to 3-mm female midge is a vicious biter, attacking in swarms in the morning and late afternoon, resulting in multiple tiny punctures.

The bites cause immediate painful papulovesicular lesions. Sensitized victims might develop an erythematous papule, indurated nodule, and urticaria. Bites from midges have also been reported to cause symptoms of rhinitis and bronchospasm.

Environmental reduction of midges is difficult because the larvae and pupal stages are ground dwelling, and the metamorphosis into adults occur at irregular intervals.

Biting midges prefer certain human hosts determined by body odor, with non-attractive people producing natural “repellents”. In areas where midges are found, they are abundant at heights of 1 to 4 meters above ground and hence bite taller people first. [12]The Strong association between the probability of bite and increasing height in men and body mass index in women has been shown.[12]

This study found no association between bites and eating strongly flavored foods (garlic, chili, and onion), contrary to popular belief that garlic makes one less attractive to biting insects. Bites may manifest as IgE-mediated urticaria or as presumably delayed-type reactions with papules, ulcers, or bullae persisting for weeks.

FLEAS[13]

These small, wingless insects live as parasites on birds and mammals. Domestic animals such as cats, dogs, and birds bring fleas into households. Newly emerging fleas become an obligate parasite once on the host.

Fleas feed by piercing the skin of the host to extract capillary blood. Saliva is introduced as an anticoagulant. Fleas can survive a remarkably long time without a host. In the absence of a host, fleas become very aggressive, provoking severe attacks on individuals moving into an empty home previously occupied by pets. Bites from fleas are typically below the knee, especially around the ankles.

Hypersensitivity is reported and, as in other causes of papular urticaria, symptoms are more common in young children. Like mosquitoes, the human host appears to become desensitized. Most flea bites resolve without treatment. Secondary infection might occur, requiring topical or systemic antibiotics.

Pruritis can be treated with oral antihistamines. Local therapy with potent, class 1, topical steroids might also be helpful. Effective flea elimination requires removal of all adult fleas as well as immature fleas. Flea bites produce maculopapular or papular rashes and severe pruritus (pulicosis).[13]

BUGS:[14]

Bugs are insects of the order Hemiptera with a common arrangement of sucking mouthparts; their hindwings are smaller than forewings.

All bugs of family Cimicidae are flattened, oval and have no hind wings; the front wings are vestigial, hence they do not fly. Adult bedbugs are about 5 mm long and may be confused with booklice, carpet beetles, and small cockroaches.

Bedbugs

Common species found in India are common bedbug and tropical bedbug. Bedbug bites are known as cimicosis. On first exposure, most individuals do not develop lesions.

With further bites, most develop an obvious skin reaction and latency for previously reacting persons decreases substantially. Few may not be sensitized even after repeated exposures as happened in a voluntarily-exposed researcher. [14]

Three salivary proteins of bedbugs, a nitric oxide–liberating heme protein (nitrophorin), a 17-kDa anticoagulant (Factor X), and a 40-kDa apyrase-like nucleotide-binding enzyme, may be important immunologically.

Bedbug infestation is facilitated by poor sanitation, overcrowding of residences and trade in second-hand furniture. Infestation in high turnover locations (hotel rooms, school hostels) may spread the disease, bedbugs being transferred with luggage to homes. Bedbugs avoid light and feed at night.[14]

The patient develops an itch or a barely visible punctum. This, if not abraded, resolves within a week. Other lesions are pruritic, usually painless, erythematous macules, papules, nodules, urticarial wheals, and blisters.[14]

Bullous rashes occurring days later may represent the late-phase response of IgE-mediated hypersensitivity to salivary protein. Common sites are arms, shoulders, and legs. Bites may produce anxiety, insomnia or delusions in a cured patient. Heavy infestation may cause significant blood loss and anemia in children.

Rare systemic reactions include generalized urticaria, asthma, and anaphylaxis. Bedbugs are suspected to transmit ≥40 human pathogens,[14] however,

there is no proven case. Exposing suspected infested household materials to sunlight has little effect as bedbugs move away to dark crevices.

Mexican chicken bugs

Bites of Mexican chicken bugs *Haematosiphon inodorus* (haematosiphoniasis) produce polymorphic lesions (wheals, papules, vesicles, pustules, and scabs). [63]

Kissing bugs

Bites of kissing bugs (*Triatoma sanguisuga*) are painless, allowing them to feed undisturbed. Initial bites produce a little reaction, with repeated exposure reactions ranging from pruritic papules with central punctum to hemorrhagic nodules and bullae may occur. Patients have multiple clustered bites, especially on the face, hence the name kissing bugs.[15]

After additional bites, the reaction may "accelerate" with local to diffuse urticaria and even erythema multiforme. Rarely anaphylactic reaction may occur, usually as urticaria or angioedema.

Often insects defecate while feeding, and parasite-laden feces from infected bugs are a source of *Trypanosoma cruzi*, the causative organism of Chagas' disease.[15]

Other insects of subfamily Triatominae may also transmit *T. cruzi*. Kissing bug bite on the face may produce Romaña sign, consisting of unilateral swelling of the eye at the site of initial infection with *T. cruzi* with localized lymphadenopathy. Swelling persists for weeks. The acute stage of Chagas is followed by indeterminate stage lasting ≥10 years.[15] Although considered pathognomonic of *T. cruzi* infection, the sign may occur after a bite in absence of *T. Cruze* transmission.

Even though the Insect bite reactions are common, the exact data on their incidence is not available in India. The incidence statistics reported by various sporadic studies conducted Children <14 years of age in dermatology outpatient clinic in Pondicherry had a prevalence of 5.3%. Children <5 years of age attending skin outpatient clinic in Calcutta had 10.6% prevalence of papular urticaria, with seasonal variation (rainy season 16.7%, summer 6.7%, winter 5.8%).

URTICARIA:

Urticaria is described by the quick manifestation of wheals and this may be accompanied by angioedema. A wheal has three typical features which comprise:

I. A centrally located swelling of varying size, almost always encircled by reflex erythema;
II. Accompanied with itching or burning and;
III. Its transient nature, where the skin returns to normal appearance within 1 hour to 24hours.[16]

The transient nature of urticaria is an important characteristic of urticarial lesions. Each individual wheal classically persists for less than a day.

In individuals suffering from physical urticaria, each individual wheal may last less than an hour. The typical urticarial lesion on physical examination is a pale-to-red and well-demarcated papule or plaque.

The shape of the lesions may be annular, serpiginous, generalized, round or oval. The urticarial lesions resolve without any post-inflammatory pigmentation or scaling.

The physical examination should focus on:

- Primary lesion characteristics: The lesions could be are edematous, erythematous papules or plaques. They have a pale center (wheal) surrounded by erythema (flare)
- Lesion distribution: Urticarial lesions can be generalized or localized.
- Lesion color: The lesions appear pale to red depending on the skin tone of the patient.
- Differentiating the types of urticaria:
    - Symptomatic dermographism is tested by stroking the skin firmly;
    - Cholinergic urticaria can be confirmed using exercise testing;
    - Cold urticaria can be tested with the application of a plastic bag filled with melting ice cubes for 5 minutes (assess for wheal response 10 min after removal of the plastic bag filled with ice)

Urticaria is not classified a life-threatening disease. Although urticaria is not life-threatening, there is evidence of negative impact among patients suffering, on their quality of life. [17]

In a conducted study by O'Donnell et al, the quality of life of daily living, including social interactions, work aptitude and quality of rest, were similar to patients suffering from heart disease.[18]

These findings were further established by an ensuing study in France and Germany. The results submitted was advising that the treating physicians should be encouraged to elaborate on the quality of life aspect of chronic urticaria with suffering patients. [19]

The type of reaction triggered by an insect bite depends on earlier exposure. An allergic reaction is developed following repeated insect bites pronounced by cutaneous manifestations. The nature of the pharmacologically active substance present in the insect bite determines the type of allergic skin reactions like wheal, erythema, bulla, vesicle or hemorrhagic nodule.

Examples of the pharmacologically active substances are hyaluronidase, proteases, histamine, and kinins etc.[20] Recurrent pruritic papules or vesicles are the classic presentations of papular urticaria accompanied with varying degrees of local edema.[21]

The most common presentation is urticarial papules followed by vesicular lesions. It is not uncommon for patients to present with urticaria as single isolated lesions.

PAPULAR URTICARIA:

Papular urticaria is a manifestation of recurrent pruritic papules or vesicles and varying degrees of local edema. Papular urticaria is a common childhood disorder and often distressing which is manifested by persistent or recurrent papules that are caused by sensitivity reaction to the bites of arthropods like mosquitoes, fleas, bedbugs, or other insects.

The reactive individual papules surround a wheal, which always often have a central punctum. The histopathological changes comprises of mild subepidermal edema, extravasation of erythrocytes, interstitial eosinophils, and exocytosis of lymphocytes.

Papular urticaria is characteristically a clinically challenging condition, especially during spring and summer months. These pruritic papules and papulovesicles are symmetrically distributed. Erosions and ulcerations result on account to itching. Pyoderma is common. These lesions occur in crops. [22]

Papular urticaria is the outcome of hypersensitivity (id reaction) to bites certain insect bites such as mosquitoes gnats, fleas, mites, and bedbugs.[1] Reactions are the result of a hypersensitivity reaction to biting, stinging, or urticating insects. The severity of the reaction is related to the host response to the salivary or contactant proteins.

Children are predisposed to papular urticaria; this is a reflection of immune mechanisms and/or behaviors that facilitate the encounters with the urticating insects. There is a seasonal predilection during spring and summer, although perennial exacerbations also occur.

Typically, papules are grouped in clusters on exposed areas, particularly extensor surfaces of extremities and constricting areas such as the tops of socks and around waistbands.

In some cases, papules follow a vascular distribution. It has also been postulated that papules around constricted areas might represent the effects of local factors, such as external pressure, which might result in a slowing of blood flow, thereby enhancing precipitation of immune complexes.

Papules might also have a more diffuse, generalized distribution involving the torso, neck, and face. The distribution of lesions serves as an important clue in identifying the culprit arthropod. Papules are erythematous, ranging from 3 to 10 mm. In the clinical setting, lesions are often excoriated and secondarily infected, contributing to the characteristic intense pruritis.

Perennial or seasonal exacerbations are common and are presumed to be associated with re-exposure to the offending arthropod. Recurrence of papular urticaria with re-exposure seems to lessen in adolescence and adulthood. This might reflect the development of immune tolerance toward the antigenic proteins[23]

PATHOPHYSIOLOGY OF PAPULAR URTICARIA:

The pathogenesis and exact immune mechanisms of papular urticaria remain somewhat unclear.

Heng *et al*. reported granular deposits in the superficial dermal vessels in three subjects with papular urticaria, suggesting that immune complexes with complement activation through the classical pathway might be involved in the pathogenesis.[23]

A subsequent study by Jordaan and Schneider [1]of 30 patients with papular urticaria failed to demonstrate granular deposition. Immunochemistry results revealed abundant T lymphocytes and macrophages.

Yoshikawa reported the histology of lesions produced by *Chelacaropsis* spp. mites in six subjects. After 48 to 72 hours of exposure, biopsies of lesions revealed perivascular aggregation of mononuclear cells and slight edema of the papillary dermis [23]**.** Although the lesions and pattern of papular urticaria are characteristic, other conditions based on presentation and/or histologic features should be considered.

IV.HUMAN CUTANEOUS LPR :

Following antigen challenge in the skin, wheal and flare reactions develop almost immediately and are characterized by a central area of pale swelling surrounded by a halo of erythema.

This macroscopic response, which is intensely pruritic, peaks in 10-15 minutes and usually resolves within 30-60 minutes. Alternatively, the immediate response may evolve into LPR characterized by burning, pruritis, erythema, induration, and warmth. LPR generally peak at 6-8 hours and are usually macroscopically resolved by 24 hours.

Skin testing in individuals may result in isolated immediate reactions, isolated delayed reactions, or dual reactions with a respective incidence of 20%, 14%, and 66-85%.

The intensity of the clinically apparent LPR appears to correlate directly with the intensity of the immediate reaction, although not all skin test-positive individuals manifest LPR. There is a direct correlation between the intensity of both the immediate and late clinical responses in patients who develop LPR; however, following skin testing of allergic subjects, histologic analyses of skin test sites 8 or 24 hours later do not differentiate between individuals who do or do not develop LPR.

Thus, patients who are skin tested with specific allergen and who manifest an immediate reaction will all histologically develop inflammatory reactions, and the intensity of these reactions bears no relationship to the clinical manifestations of LPR.

By contrast, skin testing with histamine, irrelevant antigens in allergic subjects, or pollen extracts in non allergic subjects fails to induce histologic evidence of inflammation.

Therefore, cutaneous immediate hypersensitivity responses lead to tissue inflammation, which may be accompanied by clinical signs in some but not all patients.

By definition, any substance that is capable of producing mast cell degranulation has the potential for inducing LPR. These agents include various antigens, such as ragweed, grass, and tree pollens; molds, cat and dog dander; feathers; dust and *Dermatophagoides pteronyssis*(house dust mite); *Bacillus subtilis* enzyme preparations; and insulin.

These data, further supported by the demonstration that LPR can be passively transferred to humans by affinity chromatographically purified antigen-specific IgE antibody, strongly support a central role for the mast cell in the production of LPR.

Pathogenesis of LPR:

The type I IgE-mediated hypersensitivity reaction presents with the characteristic wheal and flare reaction on the human skin. This reaction was noted to develop rapidly after injection of antigen, the hypersensitivity peaks in 10-20 min, and subsequently, subsides within a few hours. The reaction was carefully observed for longer periods. In many instances, it has been observed that a late inflammatory response is noticed the same site.

This late reaction is quite distinctive in appearance from the primary reaction. The observations of these late phase reactions have been observed and documented for many years there has been a certain amount of speculation and ambiguity revolving around their importance.

Pepys and his colleagues conducted a study to prove the significance focusing on the importance of the dual nature of the skin reactions. [24]

The immediate reaction is the result of IgE induced mast cell activation. This is followed by the late phase reaction that is noticed anywhere from 2 to 4 hours following the immediate response.

The late phase reactions characterized by the attraction of leukocytes, including neutrophils, eosinophils, basophils, and CD4+ T cells. The mast cell activation is inclusive of stimulation of pro-inflammatory mediators such as cytokines, including tumor necrosis factor (TNF). The stimulation of pro-

inflammatory mediators up-regulates endothelial expression of leukocyte adhesion molecules such as E-selectin and intercellular adhesion molecule-1 (ICAM-1). [25]

Induction of adhesion molecules is caused because of the degranulation of mast cells on the vascular endothelial cells, which stimulate the aggregation of leukocytes in the tissue in response to inflammation. Similar to the neutrophils, the endothelial cells that express E-selectins attract the eosinophils. The difference from neutrophils though is the eosinophils express Very Late Antigen 4 (VLA-4) and CD49dCD29 complex. This enables the adherence to the endothelial cells with the vascular cell adhesion molecule-1 (VCAM-1), a VAL-4 ligand.

The activated mast cells or the Th2-type cells produce IL-5. IL-5 is a chemo-attractant to the eosinophils. The infiltration of attracted eosinophils into the inflamed tissue is dependent on other chemo-attractants. They are lipid mediators, eotaxin, PAF and LTB4, and the complement product C5a. The activated eosinophils generate and release inflammation-causing mediators.

The early phases of leukocytoclastic vasculitis exhibit similar inflammatory mechanisms.[26] An eosinophil is identified to be the effector cell in the degranulation of mast cell phase in asthma similar to that in the late-phase reaction. The use of corticosteroid inhalational therapy is showing better result among patients suffering from asthma.

The mechanism of the reactions on the skin due to allergy is reconsidered with the knowledge of the late phase reaction because in a small number of persistent urticarial lesions the presence of inflammatory leukocytic infiltration has been observed. [3, 27]

The biphasic response is noticed in individuals suffering from AD after the injection of allergens intradermal. They exhibit the immediate reaction described above but between the 6 to 24 hours they exhibit the late phase reaction. This is characteristically expressed erythema, pruritus, edema, induration and skin thickening. The sequence of the chemoattractants produced and the inflammatory cells infiltration causes this late phase reaction. [24]

The initial 6 hours is witnessed with the infiltration of eosinophils, neutrophils, and basophils, which is followed by an influx of mononuclear cells inclusive of memory T cells. This scheme of infiltration of inflammatory cells is similar to the sequence seen in skin lesions of the AD. This suggests that the late phase reaction is clinically similar to disease occurring naturally than as a consequence of the immediate or primary reaction. [28]

Based on these observations, it is possible to suggest the following pathogenesis for LPR. Mast cell degranulation leads to the rapid appearance of many mediators, which cause cutaneous vasodilation, vascular permeability, and the initial attraction of polymorphonuclear leukocytes.

It can be anticipated that the effects of this group of primary and secondary mediators will be largely dissipated within minutes to hours. The mast cell granule matrix, however, provides an additional continuing source of mediators appearing over hours. The resultant neutrophil infiltration appears to be necessary for the subsequent mononuclear cell inflammatory response.

Histopathology of LPR:

In general, the histologic analysis of LPR reveals the presence of a mixed cellular infiltrate including lymphocytes, macrophages, neutrophils, basophils, and eosinophils. The relative proportions of each cell type in LPR vary considerably and may reflect a number of variables.

For instance, the number of eosinophils observed appears to be influenced by the type of inducing stimulus, the time of study and the clinical profile of the patient. Eosinophils may comprise up to 49% of the infiltrating cells in 8-hour biopsy specimens.

Basophils, although not a prominent feature of LPR (<10%), have been noted to appear in increased amounts in skin window studies of allergic subjects following allergen challenge.

The cutaneous inflammatory reaction may be so intense histologically as to involve both the perivascular and interstitial cellular space. Edema formation and vascular changes are also components of microscopic LPR.

The vascular changes include vasodilation, perivascular cellular infiltration, and endothelial hyalinization with some hemorrhage and necrosis. Intense vasculitis with hemorrhage seen in Arthus reactions is not a prominent feature.

Although the mast cell plays a primary role in the development of LPR, the precise mechanisms involved are still being unraveled. The diverse chemical mediators of the mast cell granule afford it the capability of orchestrating numerous tissue effects over a prolonged time period, including such actions as smooth muscle constriction, vascular contraction or dilation, increased vascular permeability, chemotaxis of eosinophils and neutrophils, promotion of fibrinolysis, and generation of kallikrein activity, among others.

Mast cell secretary reactions lead to granule discharge and the release and/or generation of the mediators of allergy. Histamine is undoubtedly the best-known mediator contained within the mast cell granule. When injected intradermal, it reproduces the classic wheal and flare reaction and has multiple other actions, including chemotactic properties.

Despite these capabilities, however, studies have demonstrated that histamine, when injected alone or in combination with an antigen, does not produce LPR.

These data supported further by the observation that LPR cannot be induced by cutaneous injections of bradykinin and prostaglandin E, strongly suggest that alterations in vascular permeability are not by themselves primary factors in the induction of LPR.

Studies on late phase reaction in papular urticaria:

A study conducted at the Combined Military Hospital, Abbottabad showed 1.99% patients were having papular urticaria. 71.8% patients in the study were below 12 years of age and 28.2% were above 12 years. Age of the patients up to 12 years ranged from 4 months to 12 years with a mean of 3.63 and that of patients over 12 years ranged from 13-38 years with a mean of 23.44. The total number of children below the age of 12 years having various dermatological problems was 10.34% and 13.86% out of those had papular urticaria. Out of 1.99% (280 of 14019) patients having papular urticaria, 63.6% were males. Atopic history was present in 91 (32.5%) patients.

Urticarial papules were the most common presentation (n=185, 66.1%), followed by vesicular lesions (n=64, 22.9%). Majority of the patients in this series had lesions arranged in groups. [2]

The Solely study reported its findings on late phase of the immediate wheal and flare skin reaction in 23 patients. The researchers noted that intradermal antigenic (i.e.) challenges elicited an initial wheal and flare, which usually

resolved completely, only to be followed by a late-phase reaction at the same site, characterized by diffuse erythema and edema. The late phase typically appeared by 3 to 4 hours after challenge, peaked at 6 to 12 hours, gradually subsided, and resolved by 24 hours.

Histologically and serologically, they believed it suggested an Arthus type reaction. They found the late phase was characterized by edema and a mixed cellular infiltration, predominantly lymphocytic, but also containing eosinophils, neutrophils, and basophils.

These investigators were able to elicit the late-phase response (LPR) in almost all allergic subjects, suggesting that the frequency of this reaction is much higher than previously appreciated. The interaction between antigen and mast cell-bound IgE is necessary for an allergic late phase response. [24]

Other dermatologists encountered similar cases that can be called late-phase urticaria. reported that 94% of their patients with chronic urticaria had an apparent infiltration of polynuclear leukocytes into the lesions, but two thirds of the patients had no immunocompetent deposition in the lesions. In some of these patients, eosinophil infiltration was greater than the neutrophil infiltration.[29]

Doutre reported that the histologic findings of an inflammatory reaction persisting longer than 24 to 72 hours revealed an inflammatory leukocyte infiltration. [30]

In a study conducted by Ruiz-Maldonado et al. at Mexico, it was found that papular urticaria was the most frequently observed dermatosis (16.3%) among children. They did not find significant difference among gender of the patients.[31]

Mekori et al. indicated a similarity between late-phase reaction and delayed pressure urticaria, which is noted in up to 40% of patients with acute urticaria.[32]

A study was conducted by Lakshmi C et al [33] on 14 patients presenting with clinical features of parthenium dermatitis and found to be positive for patch testing to parthenium. The study subjects included 13 males and a female aged above 30 years. 12 out of 14 patients showed a positive prick test and elevated serum IgE to different levels was found in all of them. Mean serum IgE among the study population was 1279.9 IU/ml (normal - up to 100 IU/ml). The patch test detects delayed hypersensitivity while the skin prick test (SPT) detects immediate hypersensitivity. The authors in this study have highlighted the occurrence of the late phase reaction (LPR)in the skin prick test and proposed that is mediated by newly formed mast cell mediators in concert with other inflammatory cells (eosinophils, neutrophils, lymphocytes). These mechanisms may be involved in the pathogenesis of parthenium dermatitis. Hence based on the study findings, IgE mediated late phase reaction (LPR) has been proposed as the link between immediate hypersensitivity and the development of atopic eczematous skin which histologically more closely resembles delayed-type hypersensitivity reaction by the study.

URTICARIAL VASCULITIS:

Urticarial vasculitis a form of leukocytoclastic vasculitis defined clinically by urticarial wheals that tend to be painful or to cause a burning sensation, last longer than 24 hours, and resolve with purpura.

It is often associated with hypocomplementemia and autoimmune disorders, primarily systemic lupus erythematosus. Those patients with serum hypocomplementemia, in particular, are more likely to have an associated autoimmune disease. The course of the disease is often chronic and must be differentiated from chronic urticaria.

Histologically, urticarial vasculitis shows evidence of small vessel damage, including endothelial swelling, necrosis, and fibrin deposition.[34]

Immune Complex Deposition

The initial event is the deposition of immune complexes and C3 in the postcapillary venules of clinically normal skin. The deposition of immunoglobulins and C3 in the clinically normal skin of patients with urticarial vasculitis is consistent with previous studies. In contrast, deposition of immunoglobulins is not seen in acute or chronic urticaria without evidence of vasculitis.[34]

Complement activation ensues. Low serum complement levels are detected in many but not all patients with urticarial vasculitis. A great deal has have been postulated about the role of complement in leukocytoclastic vasculitis. C5a is generated that may act neutrophil and eosinophil chemotactic factors. C3a, C4a, and C5a may mediate mast cell degranulation and cause vascular dilatation and leakage. C5a may also activate the clotting cascade. Ongoing complement activation then forms the membrane attack complex that may cause damage to the endothelial cell membranes.

Activation of Mast Cells:

Activation of mast cells and the release of mast cell mediators that include tumor necrosis factor a (TNF-a) may be triggered by complement or by other unknown factors. This is evidenced by decreased numbers of intact mast cells on histologic examination and by increased levels of serum TNF-a. The activation of mast cells and eosinophils early in the course most likely influences the urticarial nature of the initial lesions.

An increased number of mast cells and eosinophils with deposition of eosinophil granules have been demonstrated in urticarial wheals of long duration. In addition to TNF-a, mast cells release histamine, heparin, platelet-activating factor, neutrophil chemotactic factor A, leukotrienes, prostaglandins, tryptase, and neutral protease.

The exact role of TNF-a in vasculitis is not clearly defined but likely plays an important role. Tumor necrosis factor increases expression of intercellular adhesion molecule-1 (ICAM-1) on mast cells as well as E-selectin expression on endothelial cells.[35]

TNF-a also stimulates the arachidonic acid metabolism with the production of leukotrienes and prostaglandins. Early in the course of the disease, endothelial cells show increased expression of ICAM-1 and markedly increased expression of E-selectin. The expression of vascular cell adhesion molecule-1 (VCAM-1) is also present.

Intercellular adhesion molecule-1 is known to be constitutively expressed on endothelial cells and keratinocytes. The ICAM-1, VCAM-1, and E-selectin all show increased expression on endothelial cells in response to interleukin 1 and TNF-a.[36]

E-selectin acts as an adhesion molecule for neutrophils and skin-homing memory T cells that are lymphocyte function-associated antigen 3, CD-58, and leukocyte common antigen positive.

The marked increase in the expression of E-selectin is consistent with predominantly neutrophilic infiltrate within the first 24 hours of the lesion. A similar association between the expression of E-selectin and a predominantly neutrophilic infiltrate was noted by Sai set al.[37]

The expression of ICAM-1 is increased in inflammatory dermatoses characterized by T-cell infiltrates and may be important for transmigration of eosinophils.

The VCAM-1 acts as an adhesion molecule for lymphocytes, monocytes, and eosinophils. The minimal expression of VCAM-1 noted by Kano et al is puzzling considering the early eosinophilic infiltrate.

Influx and Activation of Eosinophils:

Third in the proposed sequence of events is the influx of eosinophils with deposition of eosinophilic peroxidase.

Mast cells produce interleukin 3, interleukin 5, and granulocyte-macrophage stimulating colony factor which acts as eosinophil chemoattractants. Eosinophils produce leukotrienes B4, C4, and D4 and platelet-activating factor, all of which increase vascular permeability.

These may play a role in the urticarial nature of the early lesions. Eosinophil granule basic proteins cause the further release of chemical mediators from mast cells. Eosinophils also release major basic protein and eosinophilic peroxidase that are toxic to endothelial cells.

However, it is unlikely that these alone cause endothelial cell necrosis as major basic protein has been found in chronic urticaria in the absence of vasculitis.

Persistent activation of mast cells:

Mast cells continue to be activated as evidenced by decreased numbers found at 10 and 24 hours of the disease's time course. However, the level of TNF-a present in the bloodstream falls with this time course.

Neutrophil influx with enzyme release and blood vessel damage:

The number of neutrophils within the infiltrate increases. Neutrophil elastase is detected, consistent with neutrophil disintegration, the release of neutrophilic enzymes, vascular damage, and eventual removal of immune complexes. Histologically we observe leukocytoclastic vasculitis and fibrin deposition.

ROLE OF SKIN PRICK TEST IN PAPULAR URTICARIA:

Skin prick testing (SPT) has been established as the most reliable method of diagnosing IgE-mediated allergic disease in a wide range of disease conditions. The inexpensive and simple nature of the test, reproducibility of the results, cost-effectiveness and immediate availability of the test results, makes it a highly useful diagnostic method. The basic physiological basis of SPT is that it provides evidence for sensitization and helps in confirmation of suspected type I allergy.

Skin test principle: [38]

The basic procedure involves delivering aqueous antigen beneath the stratum corneum and the barrier zone of the epidermis.

As the antigen combines with IgE antibody fixed to mast cells, mediator substances, particular histamine, are released from the mast cells. The mediators cause local vasodilatation and increased capillary permeability. Wheal and flare reactions appear in 15 minutes.

Precautions

Several precautions should not be observed during skin testing procedures:

1) Testing should be deferred during periods of symptoms to prevent worsening of the clinical status[38]
2) Allergens should be standardized, properly stored (2-8 ^{c}c). [38]
3) Systemic reactions can be provoked therefore Emergency treatment materials, syringes, and needles should be readily available to treat systemic reactions.[38]
4) Patients should be asked to avoid antihistamines and antidepressives preferably for the last 4 days but at least for 48hours
5) Patients should be observed for at least 30min after test for signs of systemic reactions.[39]

6) Patients should be asked to report if the reaction at the site is persistent/large/severe.

Site and appropriate placement of the tests:

Allergy skin testing may be performed on the back or the arm. The back is somewhat more reactive and provides a larger area for proper placement of tests.[40]

Skin testing on the forearm, however, has the advantage of allowing application of a tourniquet should a systemic reaction occurs.

It is generally recommended that prick tests be placed at least 3cm apart, [40]2cm to 2.5 cm apart, 1-inch apart.

Placing the tests too close together may cause overlapping wheals and resultant misinterpretation.

Time of evaluation

Skin tests are evaluated 15 to 20 minutes after antigen is applied.[41]

Recording the reading:

The longest and the orthogonal diameters are measured and the mean diameter is employed for analysis.

Interpretation of prick test:

To interpret the results properly, the physician must be aware of the many reasons for false- positive and false negative reactions.

False-negative results

The following circumstances sometimes account for negative skin prick test patients who have a strong history of clinical sensitivity:

- Improper storage: causing loss of potency of allergens.
- Improper administration: Too superficial a prick of the skin test will not allow the allergen solution to penetrate the stratum comeum and barrier zone of the epidermis.
- Inherent host factors: In general, the skin of infants and elderly persons is less reactive than that of other age groups. In the same individual, the forearm is less reactive than in the back.
- Refractory period: Soon after a systemic reaction to an allergen such as insect venom, penicillin, or food, the victim enters a refractory period during which a skin test reaction to that substance may be negative.
- Inhibiting drugs: such as antihistamines should be discontinued at least minimum 3 days before the skin testing.

Whenever skin tests are performed, histamine should be included as a positive control. If the result is negative, further testing should be

deferred. Corticosteroids, theophylline, cromolyn, Pagonists, and decongestants are not thought to be inhibitory. [42]

False-positive results[43]

When the skin test is positive, with no history of allergy to the antigen tested, any of several explanations may account for the situation.

- Nonspecific histamine release from Some food extracts, particularly from cheese, have high histamine content and cause false-positive reactions
- Morphine and codeine are examples of substances that always cause positive reactions.
- Dermographism**:** About 5% to 20% of persons will develop a raised, reddish mark, a response termed dermographism. A saline control should always be included to test for it.
- The negative control is important because it excludes the presence of dermographism; which if present makes the tests difficult to interpret.

  Wheal reaction equal to or greater than 5 mm, or 3mm. at a negative control is considered false positive. Whatever reaction occurs at the negative control site, should be considered a negative response.

- Positive control[35] ensures that the patient can mount a reaction to histamine and absence of a reaction can unmask interference by medications, decreased skin reactivity,[40] or technical problems with the procedure.[44].
- A skin test is considered false negative if the MWD of histamine is less than 3 mm or 4mm.

Quantitative assessment

The grading system used is less important than the knowledge of the limitations of one's technique.[41] Quantitative approaches are under consideration for prick test however it still has its limitations.[45].

There are a number of formats in use for grading the result. For clinical purposes, the grade[46] suggested is:

1+ Reactions with erythema and no wheal

2+ Reactions with wheal diameter < 3 mm

3+ Reactions with no pseudopodia and with a wheal diameter of 3 mm or larger

4+ Reactions with pseudopodia and with a wheal diameter of 3 mm or larger.

Another grading system[47] gives more important to erythema and grade suggested is:

1+ as erythema less than 20 mm without wheal,

2+ erythema greater than 20 mm and wheal less than 3 mm

3+ wheal greater than 3 mm with surrounding erythema.

4+ wheal greater than 3 mm with pseudopods and surrounding erythema.

A reaction 3+ and 4+ are considered positive.

Due to difficulty in determining the erythema in the black or deeply pigmented patient,[45] and often it is too vague for precise measurement.

Furthermore, erythema is not clearly indicative of an immunologic reaction.[44] Many authors have recommended wheal alone for comparing the results.[42].

To grade reactivity, a normal saline test ("negative control") and histamine test ("positive control") are used and the same way as the antigen.

Most test results are considered positive if the wheat diameter exceeds that of the saline control by at least 3mm indicating sensitization to the allergen.[41, 48]. Others take reactions more than 3mm as positive without considering the response at negative control.[49].

Any reaction that is more than twice the reaction at the negative control site can be considered significant.

A positive reaction consists of an urticarial wheal at least half the size of the histamine positive control. A positive skin test result was defined as a wheal diameter greater or equal to the histamine control.[50]. A reaction was positive if the wheal diameter was 2mm larger than the wheal diameter in the negative control test.

Reproducibility

Reproducibility of skin test results among experienced physicians is likely to be very good if standardized extracts of known potency are used.

The mean wheal diameter (WVD) of histamine has been used for comparing the reproducibility for each device. The state of atopy itself has previously been demonstrated not to influence the size of histamine whealing.

Coefficients of variation are an accurate assessment of precision or reproducibility. CV, a measure of how much reaction sizes deviate from the mean, is a valuable determinant of precision in any assay procedure." The level of precision is inversely proportional to the CV value; that is, low CV- high precision, high CV-low precision.

Potential adverse effects of prick test

Prick testing is quite safe since the very small amount of antigen is introduced into the skin. The potential for anaphylaxis dictates that specially trained personnel should perform skin tests only under medical supervision with equipment for resuscitation.[35]

An adverse reaction to the skin test is defined as any of the following:

i. Anaphylaxis (fall in blood pressure > 30mm Hg accompanied by hives, chest tightness, wheezing, angioedema, stridor or flushing);

ii. Generalized urticarial, sneezing, wheezing, angioedema

iii. Large local wheal and flare reaction at the site greater than 5 cm in diameter.

The advantage of prick test:

Skin test techniques share the characteristics of simplicity, rapidity of performance.

1. Identification of the allergic response directly from the patient's skin.
2. An additional benefit of prick test is a low risk of systemic reaction.
3. The test is relatively inexpensive as the test antigens are stable in 50% glycerin solution.

4. The greatest benefit of the prick test is the close contact between patient and physician or all dermatologist.[45.]

5. Low cost.

The disadvantage of prick test:

1. In spite of the accuracy in skin testing, about 10% of the positive findings will not correlate with the history.[45]
2. In addition to the problem of discomfort, skin test results are subject to variation from a number of factors that affect the skin reactivity such usage, disease status, dermatographism, drugs, and prior immunotherapy.

TREATMENT OF LPR:

Basing on the pathogenic mechanisms identified various pharmacological agents have been proposed to have a role in the treatment of LPR.

These agents include drugs belonging to various categories, including mast cell stabilizers, anti-histaminic drugs, beta-2 agonists, anti-inflammatory agents, and steroids.

Cromolyn sodium: An agent that interferes with mast cell degranulation was proven to prevent both early and late pulmonary allergic responses. The lack of systemic absorption of cromolyn sodium in significant quantities in man has precluded analysis of its activity in cutaneous LPR.

Lodoxamide ethyl: A drug possessing cromolyn-like properties, is systemically absorbed in effective quantities when administered orally. Interestingly, while this drug is able to inhibit allergic bronchial responses to inhaled antigens, it has no effect on the development of immediate and late skin responses following cutaneous antigen challenge.

These data suggest that skin mast cells may differ in their response to cromolyn-like drugs in comparison to lung mast cells. Orally administered terbutaline, a selective beta-2 agonist, does not consistently affect immediate skin test responses.

In contrast, local administration of terbutaline inhibits the immediate cutaneous response to anti-IgE. In addition, it partially reduces but does not totally abolish the late phase reaction.

Anti-Histaminic drugs: Smith and co-workers[51] reported that systemically administered H-1 antihistamines significantly attenuated immediate cutaneous allergic responses, while H-2 antihistamines had no effect. Neither agent alone affected LPR.

However, the combination of H-1 and H-2 antihistamines increased the ability of the H-1 drug to block the immediate response and completely obliterated the LPR in most subjects. These observations, however, are in contrast with observations made by other investigators regarding the relative unimportance of histamine in LPR.

In some animal studies LPR induced by either anti-IgE or mast cell granules was unaffected by H-1 antihistamines except at very high concentrations and was unaffected by H-2 antihistamines; however, it was significantly attenuated by the combination of H-1 and H-2 antihistamines.

The mechanism proposed for the therapeutic effect of this combination was by influencing the vascular responses produced during the immediate allergic reaction.

Aspirin: A cyclooxygenase pathway inhibitor, is ineffective in preventing LPR. Moreover, topical application of the 5%-indomethacin cream applied either a one-half hour before or seven hours after intradermal allergen challenge, reduces only the intensity of the initial erythema while having little effect on either the erythema or induration of the fully developed LPR.

These results suggest that prostaglandins, thought to participate in the increased vascular permeability accompanying various stages of inflammation, are unlikely to play a major role in the expression of LPR.

Corticosteroids: [52] Studies have demonstrated that steroids can prevent late phase cutaneous and pulmonary responses in man. Animal experiments have proved that LPR induced by isolated mast cell granules is also significantly attenuated by corticosteroid treatment.

The precise mechanisms by which steroids affect LPR are not clear but the following mechanisms were proposed. [53]

- Interference with histamine synthesis
- Prevention of prostaglandin formation,
- potentiation of beta-adrenergic-stimulated cyclic AMP accumulation
- Reduction of vascular permeability,
- Suppression of leukocyte adherence to the endothelium of blood vessels,
- Modulation of cellular responses to chemotactic stimuli.

MATERIALS AND METHODS

Study design:

The current study was hospital-based prospective observational study

Study setting:

The study was conducted in the Department of Dermatology, PSG, Coimbatore

Study population:

The study population was included all the subjects who are clinically diagnosed as papular urticaria

Inclusion criteria:

- Age above 10 years
- Both male and female

Exclusion criteria:

- Age less than 10 years
- History of treatment with antihistamines or oral steroids in past 3 days.
- Pregnancy and lactation
- Immunocompromised patients

Study Period:

The data collection for the study was done between July 2016 to July 2017

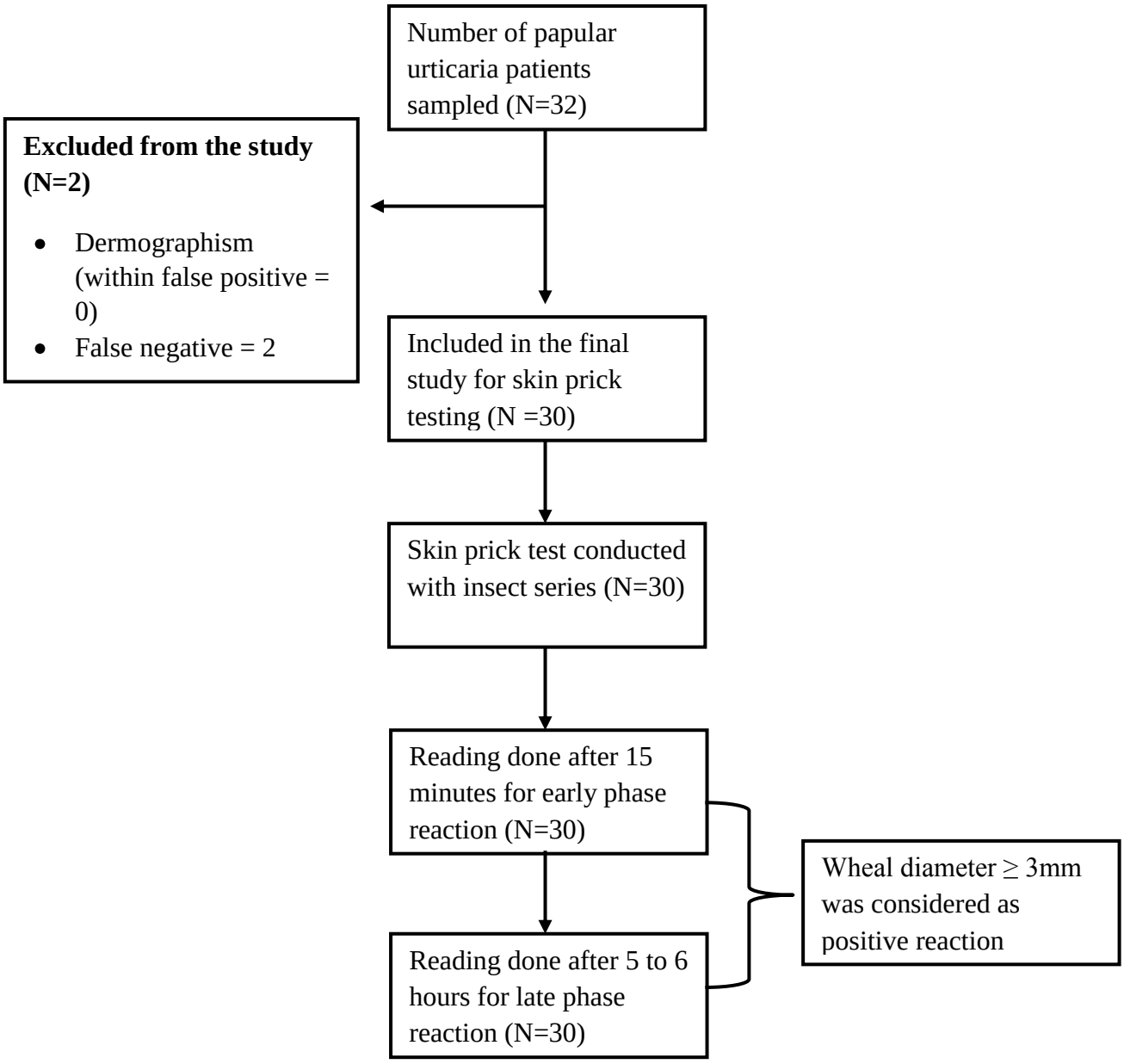
Sample Size:

A total of 30 subjects presenting with popular urticarial were included in the study

Sampling Method:

All the eligible patients were recruited consecutively by purposive sampling.

METHODOLOGY:



After obtaining the informed written consent, all the study participants were evaluated by thorough history and clinical examination to diagnose papular urticaria.

After the clinical diagnosis was confirmed, all the patients were subjected to skin testing with positive (Histamine) and negative (Normal saline) control

Negative control:

Wheal reaction equal to or greater than 5 mm, or 3mm at a negative control was considered false positive.

Positive control:

Ensures that the patient can mount a reaction to histamine and absence of a reaction can unmask interference by medications, decreased skin reactivity,[40] or technical problems with the procedure.[44]**.**

A skin test was considered false negative if the MWD of histamine is less than 3 mm.

Both false positive and false negative patients were excluded from the study

All the patients were subjected to Prick testing with insect series (commercially available and standardized antigen extracts) on the volar aspect of the forearm. The size of the wheal was measured at 15 minutes after the testing and the second and final reading was taken at 6 hours.

Prick test material

Credisol Skin Test Solutions from Creative Drug Industries, Allergology Division, Navi Mumbai are aqueous allergen extracts of insects.

Each allergen is provided in a 1.0 ml application vial, suitable for 150 tests per vial.

Allergens in the Credisol Skin Test Solutions are standardized, diafiltered and sterile, and undergo isoelectrofocusing to assure the quality of finished product.

MEDI point Blood Lancet manufactured by MEDI point, LISA (provided by Creative Drug Industries along with Credisol skin test solutions) was used for prick testing.

The lancet is made up of steel with the tip of 1mm length and has a shoulder to prevent further or deeper penetration of the tip into the dermis. Lancet is flat for better grip and has its tip directed upward on one side for easier lifting/tenting of the skin, while prick testing.

Method Of skin prick testing

- Flexor surface of forearm or arm were selected at the test site since it is easy and approachable site than back and has an advantage that tourniquet can be applied in case adverse reaction to the allergens happen.
- Test sites were marked with ballpoint pen with the respective codes of allergens.
- Precaution was taken to space the allergen at a uniform distance of 2.5 cm with the help of Spacing scale provided with Credisol skin test solutions.
- Allergens were applied aside their cue by drop technique from the application vials.
- Dab technique was avoided to prevent the contamination of the allergens through the patient's skin.
- Skin prick was made through the allergen by keeping the tip parallel to the skin surface and lifting the skin by tenting the lancet by 45 to 60 degrees. Tenting facilitates better and more entry of the allergens.
- The lancet was wiped out with a plain gauge.
- After one minute the test sites were gentle' dabbed with filter paper to remove the left out allergens.
- Each patient had a negative and positive control in the form of glycerinated saline and histamine (O. 1 %WIV) respectively.

- Care was taken to avoid a bad pick. If blood came, the test was repeated for that respective allergen. The readings were taken after 15 minutes of the prick.

Interpretation of prick test:

- Mean Wheal Diameter (MWD) was calculated as the average of the sum of longest diameter of the wheal and the orthogonal diameter to this longest diameter.
- Reactions of the allergen were compared with the negative and positive controls to minimize the false negative and false positive results.
- A reaction was considered true positive and significant only if it fulfilled the following criteria:

    1. MWD more than or equal to 3mm after reading the negative control
    2. MWD more than half of the MWD for histamine.

Ethical considerations

The study was approved by the Institutional human ethics committee of PSG medical college and Hospital, Coimbatore.

Informed written consent was obtained from all the participants after thoroughly explaining the risks and benefits involved in the study, voluntary nature of their participation.

In case of children, Informed consent was sought from the parent or guardian of the child. All data were kept confidential.

Statistical Analysis:

i. The size of the wheal at 15 minutes and at 6 to 12- hour interval was considered as the primary outcome variable.

ii. The type of immunogenic material tested was considered as a primary explanatory variable.

iii. Age and gender of the participants were other potential explanatory variables.

Descriptive statistics:

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables.

Median and IQR were used to summarize the quantitative variables with non-normal distribution.

Data was also represented using appropriate diagrams like bar diagram, pie diagram, and box plots.

Inferential statistics:

The size was the wheal association between explanatory variables and categorical outcomes was assessed by cross-tabulation and comparison of percentages. Odds ratio along with 95% CI are presented. Chi square test was used to test statistical significance. IBM SPSS version 22 was used for statistical analysis. [54]

RESULTS

A total of 30 subjects were included in the analysis

Table1: Descriptive analysis of age group in study population (N=30)

Age Group	Frequency	Percentage
Below 20	3	10.00%
21-40	16	53.33%
41-60	4	13.33%
61 and above	7	23.33%

Among the study population, 3(10%) patients were below years of age. The number of patients between 21-40 years, 41-60 years and above 61 years of age were 16(53.33%, 4(13.33%) and 7(23.33%) respectively. (Table 1 &Figure1)

Figure 1: Pie chart of age group distribution in study population (N=30)

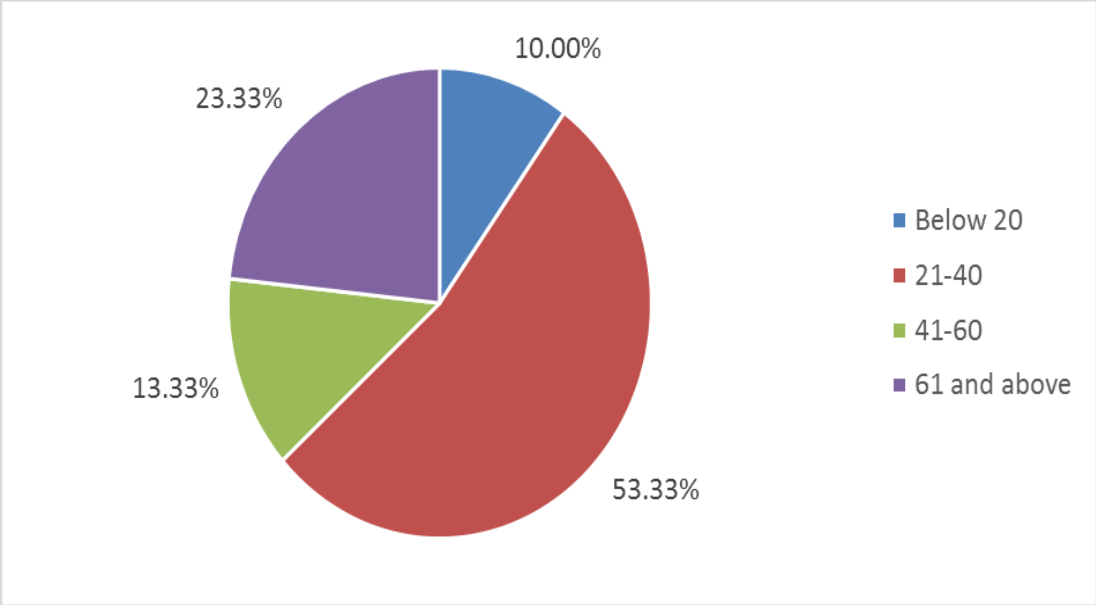


Table 2: Descriptive analysis of gender in study population (N=30)

Gender	Frequency	Percentage
Male	16	53.33%
Female	14	46.67%

Among the study population, the proportion of males were 53.33% and females were 46.67%. (Table 2 &Figure2)

Figure 2: Bar chart of gender distribution in study population (N=30)

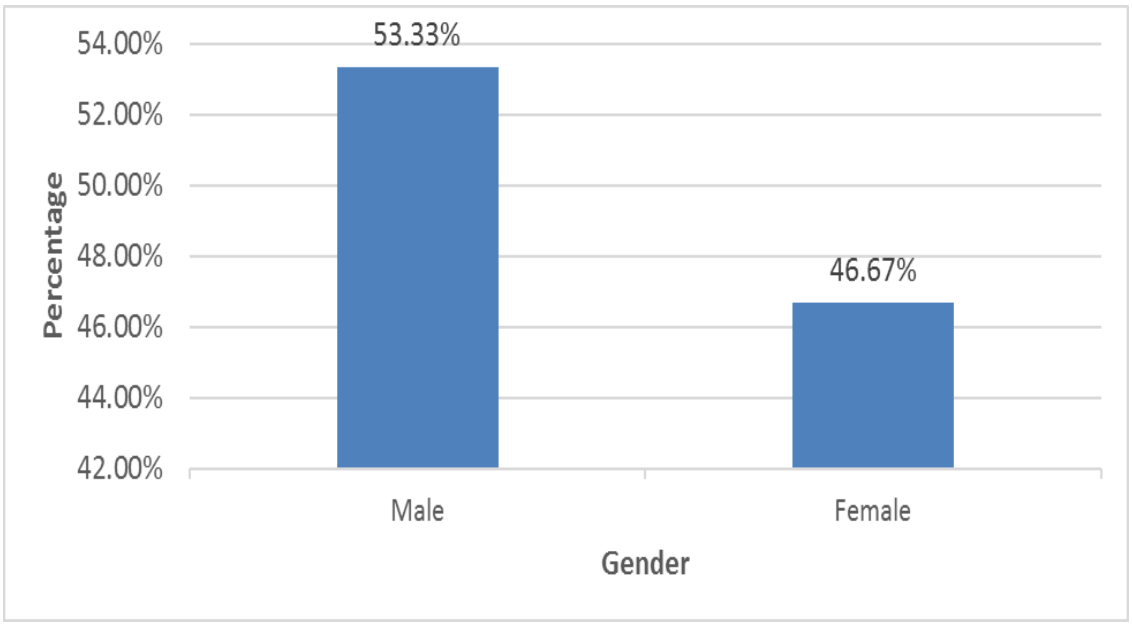


Table3: Descriptive analysis of insects in study population

Insects	Frequency	Percentage
Any EP reaction positive	30	100%
Any LP or positive	5	16.7%

Among the study population, Any Early Phase reaction positive was 30(100%) and Any Late Phase reaction positive were 5(16.7%). (table 3 & figure 3)

Figure 3: Bar chart of Insects distribution in study population

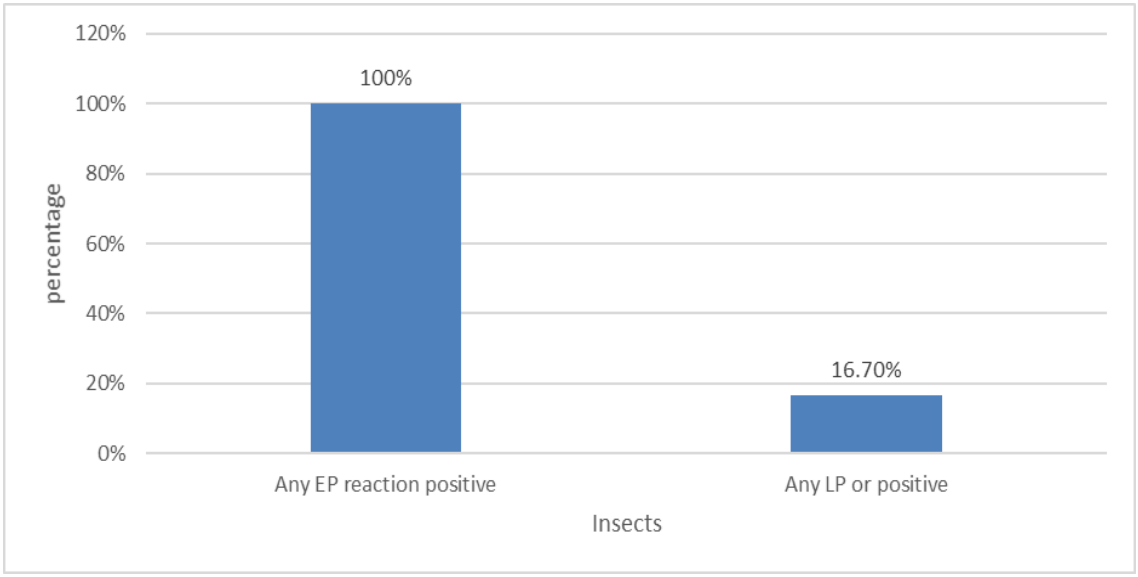


Table 4: Proportion of subject developing positive early phase Reaction (≥ 3 mm at 15minutes)

INSECTS	Frequency	Percentage
• Mite (D. Farinae)	19	63.33%
• Mite D.Petrinyssinus)	17	56.67%
• Cockroach	14	46.67%
• Mosquito	11	36.67%
• Gross hopper	10	33.33%
• Housefly	6	20.00%
• Honeybee	9	30.00%
• Cricket	7	23.33%
• Moth	6	20.00%
• Wasp	6	20.00%
• Ants	6	20.00%
• Rice weevil	4	13.33%
• Jassid	3	10.00%

Among the study population, all the subjects have developed positive early phase reaction to atleast one antigen.

The frequency of subjects developing positive early phase reaction was highest for Mite (D. Farinae) - 19 (63.33%).

The frequency of subjects developing positive early phase reaction to Mite (D.Petrinyssinus), Cockroach, Mosquito, Gross hopper, Housefly, Honeybee, Cricket, Moth, Wasp, Ants, Rice weevil and Jassid was 17(56.67%), 14(46.67%), 11(36.67%), 10(33.33%), 6(20.00%), 9(30.00%), 7(23.33%), 6(20.00%), 6(20.00%), 6(20.00%), 4(13.33%) and 3(10%) respectively.(table 4 & figure 4)

Figure 4: Bar chart of developing positive early phase Reaction (≥ 3 mm at 15minutes) Distribution in study population (N=30)

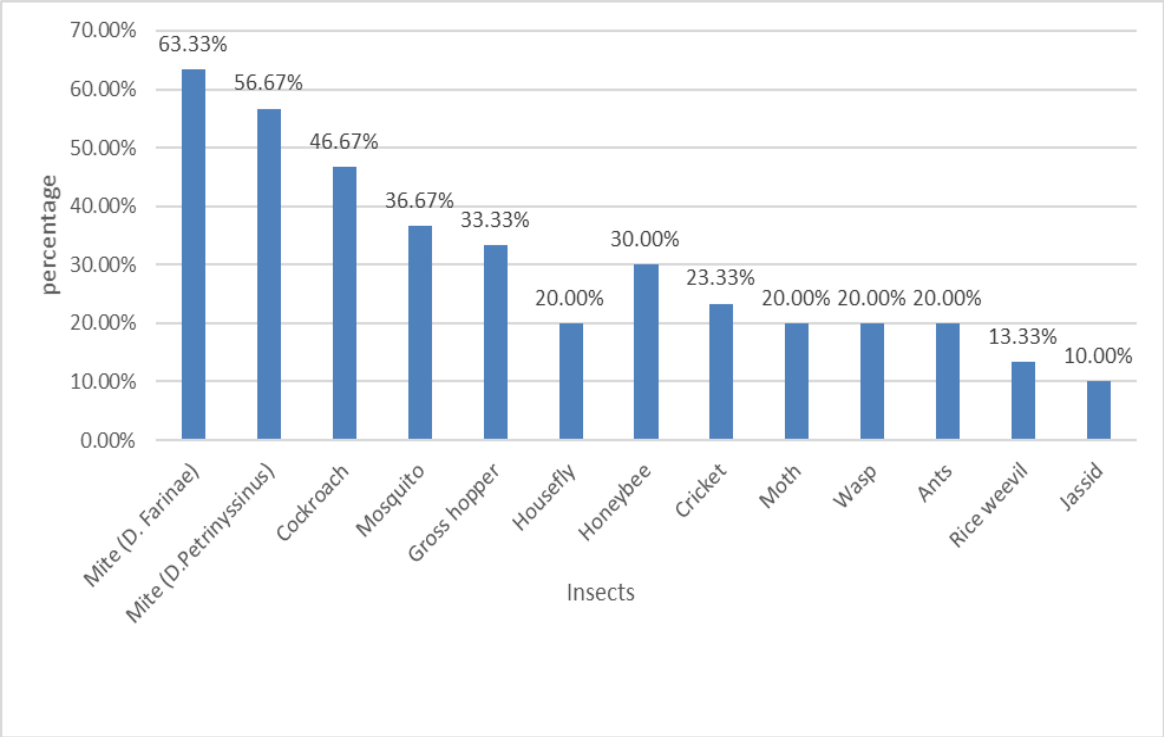


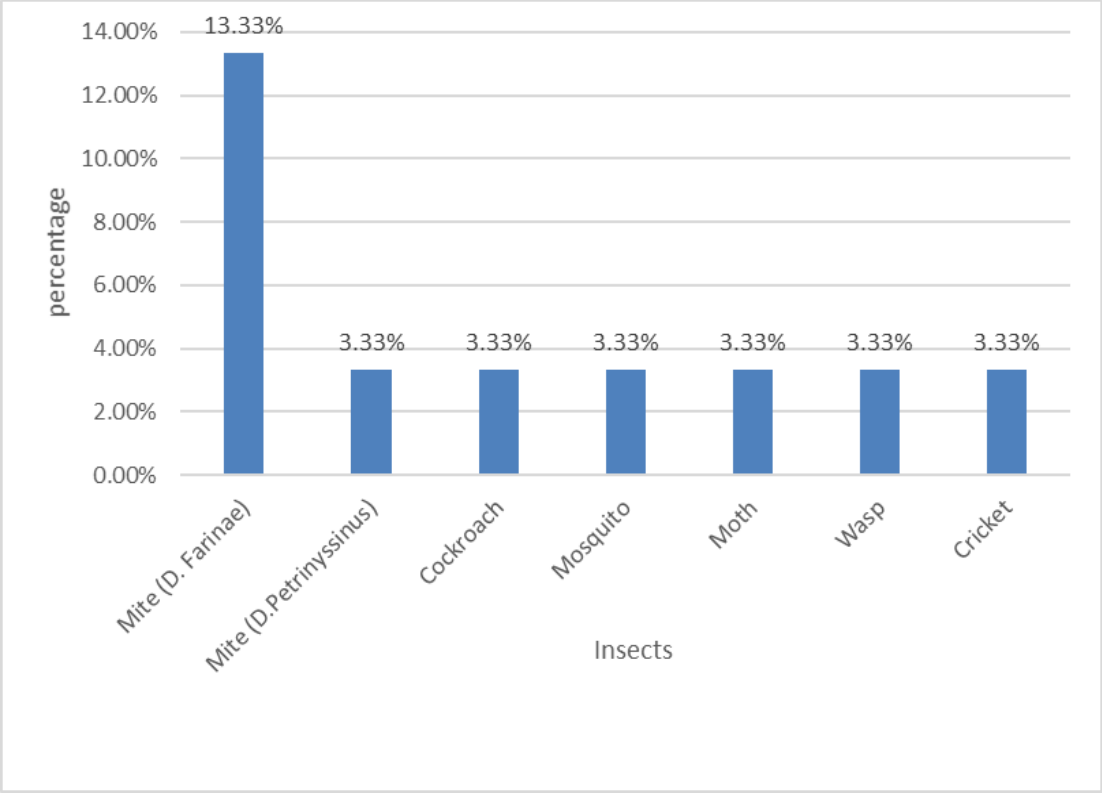
Table 5: Proportion of subject developing positive Late phase Reaction (≥ 3 mm at 6 hours)

INSECTS	Frequency	Percentage
• Mite (D. Farinae)	4	13.33%
• Mite (D.Petrinyssinus)	1	3.33%
• Cockroach	1	3.33%
• Mosquito	1	3.33%
• Moth	1	3.33%
• Wasp	1	3.33%
• Cricket	1	3.33%

The frequency of subjects developing positive Late phase reaction for Mite (D. Farinae) was 5(16.7%).

The frequency of subjects developing positive Late phase reaction for Mite (D.Petrinyssinus), Cockroach, Mosquito, Moth, Wasp and Cricket was 4(13.33%), 1(3.33%),1(3.33%), 1(3.33%), 1(3.33%), 1(3.33%) and 1(3.33%) respectively. (table 5 & figure 5)

Figure 5: Bar chart of developing positive late phase Reaction (≥ 3 mm at 6 hours) (N=30)



DISCUSSION

Papular urticaria is a common childhood disorder and in India is usually occurs due to hypersensitivity (id reaction) to certain insect bites.[1]

This hypersensitivity reaction can be caused by sensitivity reaction to the bites of arthropods like mosquitoes, fleas, bedbugs, or other insects. The reactive individual papules surround a wheal, which always often have a central punctum.[22]

The usual manifestation is an acute phase reaction following antigen challenge in the skin, immediately after the bite, the wheal and flare reactions develop almost instantly characterized by a central area of pale swelling surrounded by a halo of erythema. This macroscopic response, which is intensely pruritic, peaks in 10-15 minutes and usually resolves within 30-60 minutes.

Alternatively, the immediate response may evolve into LPR characterized by burning, pruritus, erythema, induration, and warmth. LPR generally peak at 6-8 hours and are usually macroscopically resolved by 24 hours.

The Abbottabad study identified patients naturally exposed to insect bites presenting with a papular urticarial response. The present study was done to using insect series on patients and the subsequent late phase wheal and flare response.

Author Yuka Asai conducted a case study on five patients with a late onset of acute urticaria following a bee sting. The patients were of Japanese origin and their ages ranged from 33 to 86 years. None of the study subjects had previous

allergic reaction history to bee stings. The onset of urticaria was 6–14 days after a bee sting.

Among the study group, 4 of the patients did not describe experiencing a bee sting at their presentation but the subsequent examination detected anti-bee-specific IgE antibodies. [55]

The proportion of subjects developing positive early phase Reaction (≥ 3 mm at 15minutes) in the present study to honeybee was 30% and none of them showed late phase reaction.

Among the study population, all the subjects have developed early phase response to at least one antigen in the insect series. The number of subjects developing positive early phase reaction was highest for Mite (D. Farinae) - 19 (63.33%).

The number of subjects developing positive early phase reaction for Mite (D. Petrinyssinus), Cockroach, Mosquito, Gross hopper, Housefly, Honeybee, Cricket, Moth, Wasp, Ants, Rice weevil and Jassid was 17(56.67%), 14(46.67%), 11(36.67%), 10(33.33%), 6(20.00%), 9(30.00%), 7(23.33%), 6(20.00%), 6(20.00%), 6(20.00%), 4(13.33%) and 3(10%) respectively.

Nadeem Raza et al presented 66.8% patients with the first episode of papular urticaria and 6.1%patients had periodic episodes of the eruption for more than three years' duration. 97.9% patients slept indoor, 51.1% slept on wooden bed and

mattress and 59.6% wear full sleeves while sleeping at night. Atopic history was present in 32.5% patients. The family history of insect bites was present in 26.7% patients.

Dermatological examination revealed that 64.3% patients had lesions over both exposed as well as covered parts, whereas 12.9% patients developed lesions only overexposed parts of the body. In 56.8% of patients, the number of lesions was between 6 and 15. Lesions were arranged in groups in 54.3% patients and linear distribution was evident in 10.0% patients.

Urticarial papules were the most common presentation 66.1%, followed by vesicular lesions in 22.9%. Majority of the patients in this series had lesions arranged in groups. [2]

There are no noted studies conducted on the antigenic challenges using skin pricks and subsequent study of the late phase reactions. The Solely study used intradermal antigenic challenges of types of ragweed and reported its findings on late phase of the immediate wheal and flare skin reaction in 23 patients. The researchers noted that intradermal antigenic (IgE) challenges elicited an initial wheal and flare, which usually resolved completely, only to be followed by a late-phase reaction at the same site, characterized by diffuse erythema and edema.

The late phase typically appeared by 3 to 4 hours after challenge, peaked at 6 to 12 hours, gradually subsided, and resolved by 24 hours. Histologically and serologically, they believed it suggested an Arthus type reaction. They found the late phase was characterized by edema and a mixed cellular infiltration, predominantly lymphocytic, but also containing eosinophils, neutrophils, and basophils.

These investigators were able to elicit the late-phase response (LPR) in almost all allergic subjects, suggesting that the frequency of this reaction is much higher than previously appreciated. The interaction between antigen and mast cell-bound IgE is necessary for an allergic late phase response. [24]

The present study used insect series to test the LPR to immediate response. The immediate response was highest, 63.33% with skin prick of Mite (D. Farinae) which subsequently at the 6^{th} hour 21% of them exhibited LPR.

56.67% showed an early reaction to another mite variety - Mite (D. Petrinyssinus) but the late phase reaction was exhibited in a meager 5.9% of them.

Sugita et al encountered similar cases that can be called late-phase urticaria, but unlike my study to report the presence of late phase wheal and flare response following insect series, they reported the infiltration of polymorphonuclear leukocytes into the lesions and immunocompetent deposition in the lesions with emphasis on eosinophil and neutrophil infiltration.[29]

Similar to Sugita, Doutre reported that the histologic findings of an inflammatory reaction persisting longer than 24 to 72 hours revealed an inflammatory leukocyte infiltration. [30]

A study was conducted by Lakshmi C et al [33] on 14 patients presenting with clinical features of parthenium dermatitis and found to be positive for patch testing to parthenium.

The study subjects included 13 males and a female aged above 30 years. 12 out of 14 patients showed a positive prick test and elevated serum IgE to different levels was found in all of them. Mean serum IgE among the study population was 1279.9 IU/ml (normal - up to 100 IU/ml).

The authors in this study have highlighted the occurrence of the late phase reaction (LPR)in the skin prick test and proposed that is mediated by newly formed mast cell mediators in concert with other inflammatory cells (eosinophils, neutrophils, lymphocytes). These mechanisms may be involved in the pathogenesis of parthenium dermatitis. Hence based on the study findings, IgE mediated late phase reaction (LPR) has been proposed as the link between immediate hypersensitivity and the development of atopic eczematous skin which histologically more closely resembles delayed-type hypersensitivity reaction by the study.

Basing on the pathogenic mechanisms identified various pharmacological agents have been proposed to have a role in the treatment of LPR. These agents include drugs belonging to various categories, including mast cell stabilizers, anti-histaminic drugs, beta-2 agonists, anti-inflammatory agents, and steroids. Many of these pharmacological agents were assessed for their effectiveness in animal models.

Mast cell stabilizers like Cromolyn sodium, Iodoxamide ethyl and prostaglandin inhibitors like Aspirin have been proven to have minimal benefit in late phase reaction.

Combination of anti-Histaminic drugs the combination of H-1 and H-2 antihistamines increased the ability of the H-1 drug to block the immediate response and completely obliterated the LPR in most subjects. These observations, however, are in contrast with observations made by other investigators regarding the relative unimportance of histamine in LPR.

But studies have demonstrated that steroids can prevent late phase cutaneous and pulmonary responses in man. Animal experiments have proved that LPR induced by isolated mast cell granules is also significantly attenuated by corticosteroid treatment.

The precise mechanisms by which steroids affect LPR are not clear but the following mechanisms were proposed. Interference with histamine synthesis Prevention of prostaglandin formation, potentiation of beta-adrenergic-stimulated cyclic AMP accumulation Reduction of vascular permeability, suppression of leukocyte adherence to the endothelium of blood vessels, Modulation of cellular responses to chemotactic stimuli.

CONCLUSIONS

Early phase urticarial reaction is common with many common insect antigens. Mites, followed by cockroach and mosquito antigens seem to be highly allergenic, as compared to other antigens. The late phase urticarial reaction is also seen in nearly One-sixth of the cases. Mite (DF) has caused the highest proportion of late phase positive reaction.

STRENGTHS OF THE STUDY

1. Even though there are multiple studies on early phase reaction in papular dermatitis, this study focuses on the late phase reaction. By highlighting the occurrence of late phase reaction this current study can help in drawing the attention of clinicians toward this ignored aspect of the disease.
2. The study findings may pave way for further research on the subject.

LIMITATION

1. Biopsy of the lesions and histopathological examination would have provided a more useful understanding of the pathophysiology of late phase reactions. This could not be done in the study, due to resource constraints.

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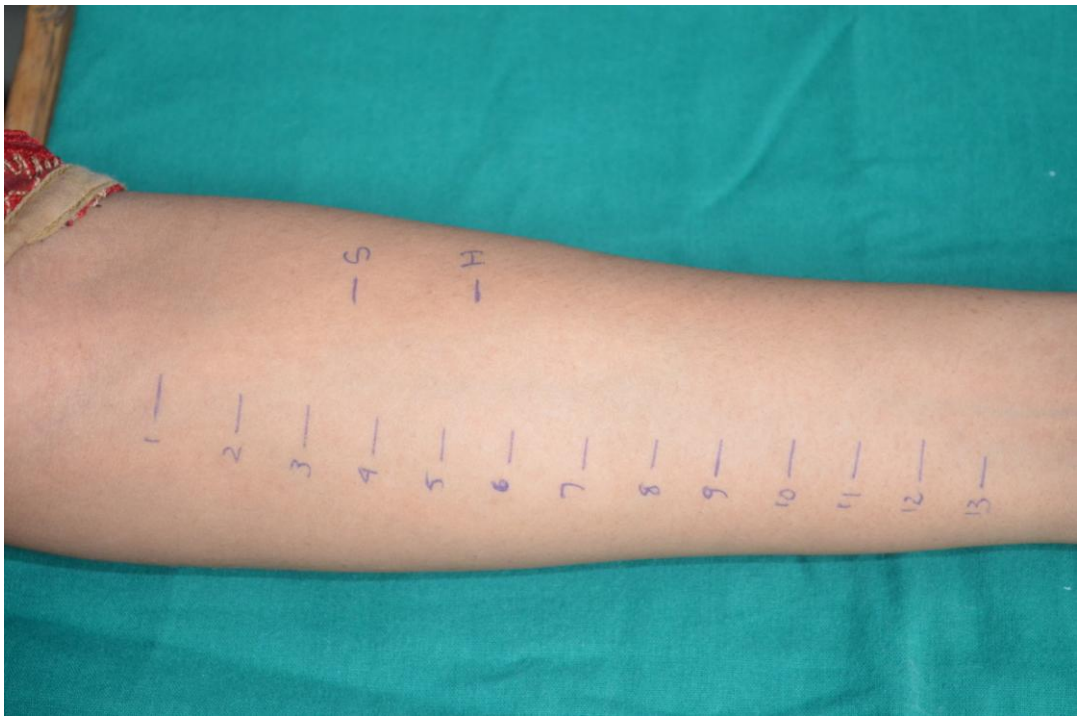
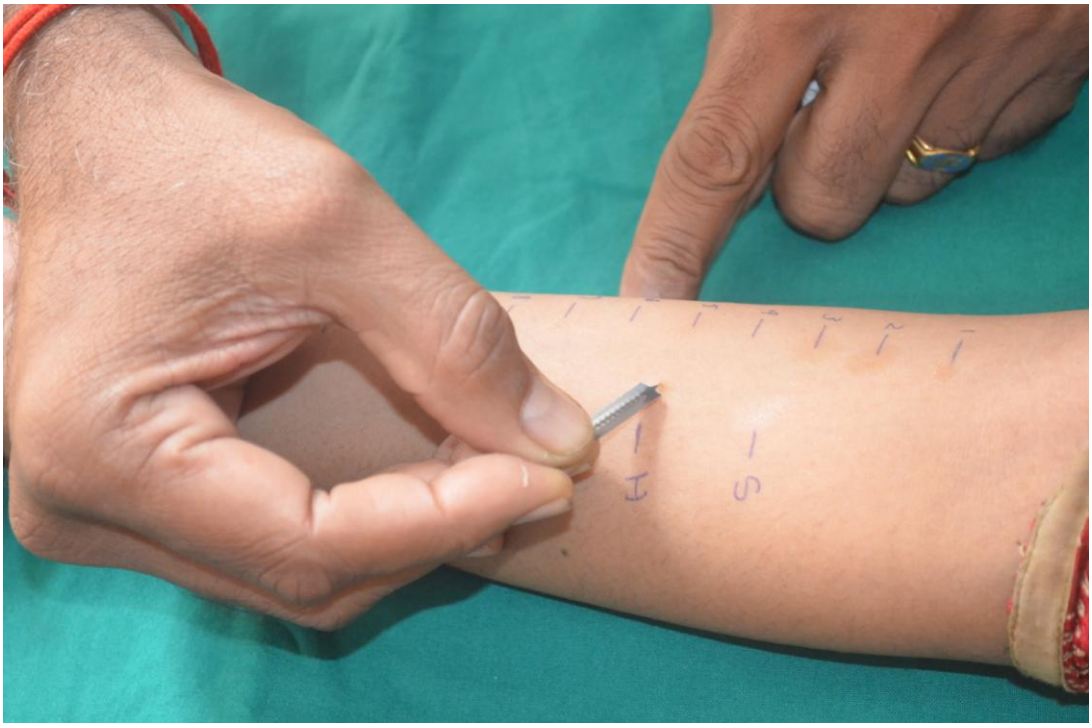
CLINICAL PHOTOGRAPHS

Credisol skin test insect antigens with MEDI point blood lancet

PRICK TESTING





PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
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To
Dr Iyshwariya Sivadasan
Postgraduate
Department of Dermatology
Guides: Dr C Shanmuga Sekar / Dr C R Srinivas
PSG IMS & R
Coimbatore

Ref: Project No.16/232

Date: July 26, 2016

Dear Dr Iyshwariya,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 05.07.2016 to conduct the research study entitled "*Prick testing in insect bite reaction*" during the IHEC meeting held on 08.07.2016.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 05.07.2016)
3. Informed consent forms (Version 1.1 dated 22.07.2016)
4. Data collection tool (Version 1 dated 05.07.2016)
5. Current CVs of Principal investigator, Co-investigators
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 08.07.2016 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

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Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

PSG HOSPITALS

Department of Dermatology

Prick Test Form

Name : Age / Sex: Date:

OP No. :

Address

Diagnosis :

INSECT

No.	Name of Allergens	After 15 min (Size of Wheal in mm)	6-12 hours Reading
1	Mite (D. Farinae)		
2	Mite (D. Petronyssinus)		
3	Cockroach		
4	Mosquito		
5	House Fly		
6	Rice Weevil		
7	Moth		
8	Wasp (Yellow)		
9	Grass hopper		
10	Ants		
11	Honey bee		
12	Cricket		
13	Jassid		
14	Histamine		
15	Saline		

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I Dr. Iyshwariya Sivadasan, am carrying out a study on the topic: "PRICK TESTING IN INSECT BITE REACTION"

as part of my research project being carried out under the aegis of the Department of Dermatology

My / our research guide is: Dr. Shanmuga Sekar.C.,

The justification for this study is: To observe for late phase reaction and plan management accordingly.

The objectives of this study are:

Primary Objective: To assess the type 1 hypersensitivity reaction – early phase (assessed at 15 minutes) and late phase (assessed at 6 hours) by prick testing with insect series in patients with papular urticaria.

Sample size: 30 patients.

Study volunteers / participants are (specify population group & age group): above 10 years of age.

Location:Patients presenting to the dermatology OPD at PSGIMS & R , Coimbatore.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration) 15 minutes.

Data collected will be stored for a period of 10 years. We will / will not use the data as part of another study.

Benefits from this study: Identifying patients with late phase reaction will help in better management of the condition.

Risks involved by participating in this study: Mild erythema and edema

How the **results** will be used: Patients showing late phase reaction may not be responsive to treatment with anti histamines and will require systemic corticosteroids or calcineurin inhibitors.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: 9840199981

Contact number of Ethics Committee Office: During Office hours: 0422 2570170 Extn.: 5818
After Office hours: 9865561463

ஒப்பதல் படிவம்

தேதி

ஐஸ்வர்யா சிவதாசன் ஆகிய நான் PSG மருத்துவக்கல்லூரியின் **தோல் பால்வினை மற்றும் தொழுநோய்** துறையின் கீழ் **ஊசி மூலம் பூச்சி கடிஒவ்வாமை கண்டறிதல்** என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி : மரு. சி.ஆர். சீனிவாஸ், மரு. சண்முகசேகர் சி.

ஆய்வு மேற்கொள்வதற்கான அடிப்படை

தாமதமாக ஏற்படக்கூடிய விளைவுகளை கண்டறிதல்.

ஆய்வின் நோக்கம்

பூச்சி கடியால் ஏற்படக்கூடிய தாமதமான விளைவுகளை கண்டறிதல்.

ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை : 5 வயதிற்கு மேல் உள்ள ஆண்களும் பெண்களும்.

ஆய்வு மேற்கொள்ளும் இடம் : பி.எஸ்.ஜி மருத்துவமனை
தோல் பால்வினை மற்றும் தொழுநோய் துறை

இந்த ஆய்வில் எங்களுடன் ஒத்துழைக்குமாறு கேட்டுக் கொள்கிறோம். நாங்கள் சில தகவல்களை இந்த ஆய்விற்காக சேகரிக்க உள்ளோம்.

ஆய்வு செய்யப்படும் முறை

பூச்சிக்கடி ஒவ்வாமை உள்ளவர்களுக்கு ஊசி மூலம் அலர்ஜி பரிசோதனை செய்யப்படும். 15 நிமிடங்களுக்குப் பிறகும், 6-12 மணிநேரத்திற்கு பிறகும் ஏற்படக்கூடிய விளைவுகள் கண்டறியப்படும்.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 3 வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப்பட மாட்டாது. எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது. இவை இரகசியமாக வைக்கப்படும்.

சுகாதார கல்வி பற்றிய விளக்கங்கள்.

இப்பகுதியானது இந்த ஆய்வில் இடம் பெறவில்லை.

ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள் :

தற்காலிகமாக சிவந்து போகுதல் மற்றும் லேசான எரிச்சல் ஏற்படலாம்.

இந்த ஆய்வின் பயன்கள்

பூச்சிக்கடி ஒவ்வாமை உள்ளவர்களுக்கு ஊசி மூலம் அலர்ஜி பரிசோதனை செய்து, அதற்கேற்ப மருத்துவ சிகிச்சை அளிக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

ஆய்விலிருந்து விலகிக் கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படமாட்டாது

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும் இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

ஆய்வாளரின் தொலைபேசி எண் 9840199981

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண். 0422-2570170 Extn. 5818

ABBREVIATIONS

ICAM-1	-	Intercellular Adhesion Molecule-1
IgE	-	Immunoglobulin E
IL	-	Interleukin
LPR	-	Late Phase Reaction
MBP	-	Major basic protein
MWD	-	Mean Wheal Diameter
PAF	-	Platelet Activating Factor
SPT	-	Skin Prick Test
TNF	-	Tumor Necrosis Factor
VCAM-1	-	Vascular Cell Adhesion Molecule-1
VLA-4	-	Very Late Antigen 4

MASTER CHART

SNO	Name	Age	Gender	MiteDF@15min	MiteDF@6h	MiteDP@15min	MiteDP@6h	Cockroach@15min	Cochroach@6h	Mosquito@15min	bsquito@6	usefly@15	pusefly@6h	weevil@15	weevi@6	oth@15m	Moth@6hr	/asp@15m	Wasp@6hr	hopper@1s	hopper@	nts@15mi	Ants@6hr	ey bee@15	ney bee@6	cket@15m	ricket@6h	assid@15m	assid@6hr	amine@15	tamine@6	pline@15m	paline@6h
1	parvathikumari	62	2	3	1	3	1	3	1	6	1	2	1	2	1	5	1	5	1	4	1	4	1	3	1	3	1	2	1	5	2	2	1
2	Sudha	31	2	6	3	3	3	2	1	2	1	3	1	2	1	4	1	2	1	2	1	2	1	3	1	4	1	3	1	5	2	3	1
3	Kumarasamy	69	1	3	1	3	1	3	1	3	1	2	1	2	1	3	1	2	1	3	1	3	1	3	1	2	2	2	2	6	3	4	2
4	Sumathi	37	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	6	2	3	1
5	Elamaran	36	1	4	1	6	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	5	2	3	1
6	Ramesh	23	1	9	2	10	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	8	3	4	1
7	Pavithra	18	2	6	1	2	1	2	1	3	1	5	1	2	1	2	1	3	1	3	1	2	1	5	1	2	1	2	1	5	2	3	1
8	Sugumar	65	1	2	2	2	2	5	2	2	1	5	1	2	1	2	1	2	1	2	1	2	1	2	1	3	1	2	1	5	4	3	2
9	Venkadesh	44	1	2	1	5	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	4	1	5	1	4	2	2	1
10	Sarasammal	73	2	2	1	2	1	2	1	2	1	2	1	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	7	2	4	1
11	Sundari	66	2	2	1	2	1	2	1	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	7.5	3	3	1
12	Merlin	20	2	2	1	2	1	2	1	2	1	2	1	3	1	2	1	2	1	2	1	3	1	2	1	3	1	2	1	4	2	2	1
13	Aswin	24	1	5	1	6	1	4	1	2	1	7	1	3	1	2	1	2	1	4	1	7	1	2	1	2	1	2	1	5	3	2	1
14	Karthikeyan	32	1	6	4	3	1	7	4	5	3	2	1	4	1	5	3	5	3	3	1	3	1	2	1	2	1	2	1	5	2	3	1
15	Suresh	32	1	6	3	5	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	5	2	3	1
16	Ravikumar	43	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	7	2	2	1	2	1	2	1	2	1	6	3	3	1
17	Pavithras	20	2	2	1	2	1	3	1	3	1	2	1	2	1	2	1	2	1	2	1	2	1	3	1	2	1	2	1	6	3	4	2
18	Loganathan	33	1	5	1	5	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	4	2	3	1
19	Ganesan	36	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	6	3	4	1
20	Poongodi	38	2	6	1	6	1	4	1	6	1	2	1	2	1	6	1	2	1	2	1	2	1	2	1	6	4	3	1	5	2	3	1
21	Selvi	40	2	6	1	2	1	2	1	3	1	5	1	2	1	2	1	3	1	3	1	2	1	5	1	2	1	2	1	5	2	3	1
22	Manju	24	2	9	2	10	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	8	3	4	1
23	Raja	27	1	6	1	2	1	2	1	3	1	5	1	2	1	2	1	3	1	3	1	2	1	5	1	2	1	2	1	5	2	3	1
24	Rajesh	32	1	2	1	2	1	3	1	3	1	2	1	2	1	2	1	2	1	2	1	2	1	3	1	2	1	2	1	6	3	4	2
25	Ravi	43	1	5	1	5	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	4	2	3	1
27	Lakshmi	38	2	3	1	3	1	3	1	6	1	2	1	2	1	5	1	5	1	4	1	4	1	3	1	3	1	2	1	5	2	2	1
28	Lenin	40	1	6	3	5	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	5	2	3	1
29	parvathikumari	69	2	9	2	10	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	8	3	4	1
30	Sarasu	70	2	4	1	6	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	5	2	3	1